REMARKS

As background, *E. coli* is a bacterium, which is known to cause diarrhea, urinary tract infections (UTI) and neonatal meningitis in humans. Some strains of this bacteria are more dangerous than other strains.

Virulent strains of *E. coli* and certain other virulent bacteria are characterized by the presence of antigens located in their cell walls ("O" antigens). These antigens are polysaccharides and vary significantly in individual bacterial species and strains. This antigen diversity is a survival mechanism, which protects the bacteria from the immune system (Lui et al (of record), page 2102, first column, first paragraph).

Usually the body will manufacture antibodies against each antigen. The body has a "memory" and can manufacture very rapidly antibodies against an antigen it has encountered before. However, it takes time for the body to manufacture antibodies to antigens it has not encountered before, which can result in infection/illness while the immune system has time to respond.

This inherent variation (the specification indicates that 166 antigens had been defined as of the effective filing date) in the antigens makes reliable identification of the antigens themselves by analytical methods very difficult, expensive and unreliable.

One method is to use polyclonal antibodies to screen for the presence of the antigens and then extrapolate to conclude that the relevant bacterial serotypes are present in the sample. This method has several problems, including the fact it cannot

identify the presence of bacterial strains with highly related forms of "O" antigens. Additionally, such methods, which profile or fingerprint the relevant bacteria to be built up using multiple components, are complicated not the least of which because of the difficulties associated with the production and purification of polyclonal antibodies on a commercial scale.

The assembly of polysaccharide "O" antigens in the bacteria is controlled by certain genes, such as transferases, polymerases and flippases (see page 6, line 35, to page 7, line 2, of the present specification). This is in contrast to genes which simply synthesize the component sugars (sugar pathway genes).

The present invention is based on the realization that the "assembly" genes are conserved in different serotypes of these virulent bacteria, and that non-pathogenic bacteria do not have the same "assembly" genes. Thus, the presence of the "assembly" genes can be used to identify indirectly the presence or absence of pathogenic bacterial serotypes in a given sample. Hence, the present invention provides a method of identifying said bacteria based on the specificity of one or more relevant oligonucleotides to one of the "assembly" genes.

The present invention provides a reliable, efficient and inexpensive method of identifying the relevant bacteria in a given sample because it does <u>not</u> rely on direct identification of the "O" antigen.

In paragraph 2, on page 2 of the Office Action, the Examiner rejects Claims 85-106 under 35 U.S.C. § 112, first paragraph as lacking written description.

Specifically, in paragraph 5, on page 4 of the Office Action, the Examiner states that the claimed assay does not comprise any method steps which seek to determine that the polysaccharide antigens are in fact present, i.e., the method only determines if a nucleic acid sequence, which is assumed to encode the antigens, is present in the sample.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

In view of the amendment to the claims (as presented in new Claims 107-128) for ease of consideration, to recite that the present invention relates to "a method of testing a sample for the presence of *E. coli* encoding bacterial polysaccharide "O"-antigen serotype O11" and "a method of testing a sample for the presence of *E. coli* encoding bacterial polysaccharide "O"-antigen *S. enterica*", Applicants believe that this aspect of the Examiner's rejection has been rendered moot.

The Examiner also contends that if one were to analyze a plasmid in *E. coli* which happens to contain one of the relevant sequences, the assay would result in a false positive signal as the nucleic acid of interest would be present, but is not found in any of the identified organisms. In this case, the Examiner contends that the nucleic acid does not have to be expressed or even be in the correct reading frame such that if expressed, that it would encode the desired antigen.

Initially, Applicants respectfully submit that the techniques employed in the claimed method are within the ordinary skill of one in the art. One of the standard laboratory texts employed in the field of DNA technology is

Sambrook et al, "Molecular Cloning: A Laboratory Manual", 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). This text outlines the basic techniques employed in the present invention.

In any event, the Examiner's comments about false positive are addressed at page 12, lines 10-17, of the present specification, wherein it teaches that cross-reaction (or false positives) can be avoided by using multiple oligomers directed to different "assembly" genes. Further, at page 13, lines 16-21, of the present specification, it is taught how to identify spurious results (see also page 18, lines 3-10, of the present specification).

In paragraph 6, on page 5 of the Office Action, the Examiner states that in the claims one is to hybridize "at least one oligonucleotide" to one of the identified sequences wherein "high stringent wash conditions" are used, and thereby only duplex structures that have hybridized to one another would have great specificity. However, the Examiner contends that in accordance with the claims, a primer pair is employed in step (b), but only one of the primers specifically hybridizes to the nucleic acid sequence. It is the Examiner's position that the specification does not provide a description on how non-specific hybridization can take place, and remain, when using the recited stringent wash conditions.

Applicants respectfully submit that the Examiner's rejection is improper since the oligonucleotide molecules are specific for, e.g., the O-antigen serotype O111. Thus, there is no non-specific hybridization. Rather, the methods of the

invention may employ probes or primers that hybridize under stringent wash conditions to a non-specific region of nucleic acid of the bacterium being tested for. This is clearly evident from a plain reading of the specification, and is discussed in detail at page 15, lines 16-21, of the present specification. Hence, any description as to how "non-specific" hybridization could occur under stringent wash conditions is clearly superfluous and contradictory in the present case.

In paragraph 7, on page 5 of the Office Action, the Examiner notes that Claims 88, 92, 96 and 100 recite a listing of nucleic acids, one of which is to be used in the claimed method. It is the Examiner's position that the specification does not describe which of these sequences hybridize specifically and which do not.

In new Claims 107-128, the wording "specifically hybridizing" has not been used even though Applicants believe that the specification supports the meaning, i.e., hybridization to the gene rather than to "extraneous" portions of the sequence (see page 15, line 21, of the present specification).

In any event, the oligonucleotides of the claims are defined in such a way that it is an implicit feature that they specifically hybridize to the gene. That is, all of the sequences of these claims (new Claims 107-128) hybridize specifically to the bacterial serotypes of interest.

In any event, Applicants respectfully disagree with the Examiner's position that the specification contains inadequate teachings as to what combinations of sequences may be used as primers. The skilled person in the field of DNA technology

clearly understands the general process for the selection of appropriate primer pairs. This is clearly outlined in the specification at pages 41-44, where the selection of numerous primer pairs is described, as well as their relative success when used in the polymerase chain reaction. Primer pairs that produced oligonucleotide fragments of an inappropriate length were considered unsuitable primer pairs. Tables 4-8 list the specific sequences of the respective forward and reverse primers used in the aforementioned primer testing. The basics of primer design are also outlined in a Rybicki, "PCR Primer Design and Reaction Optimisation", Cone et al (Eds.), Molecular Biology Techniques Manual, 3rd Ed. (1992) (a copy of which is attached hereto). This text represents a standard laboratory manual commonly referred to by those involved in PCR methodologies.

Accordingly, Applicants respectfully submit that the claims have written description support in the specification, and thus request withdrawal of the Examiner's rejection.

In paragraph 11, on page 6 of the Office Action, the Examiner rejects Claims 86, 90, 94 and 98 under 35 U.S.C. § 112, second paragraph.

Specifically, the Examiner contends that the expression "specifically hybridize" is indefinite.

As noted above, the new claims do not contain the objected to language, and thus Applicants submit this rejection has been rendered moot.

In any event, Applicants enclosed herewith a Declaration by the inventor, Professor Peter Reeves, as to what is commonly understood by this term within the technical field of the

invention. Applicants respectfully submit that this term has a clear and well-established interpretation within the field, and as such, it is unduly limiting to require Applicants to include the specific wash conditions in the claims.

In paragraph 16, on page 7 of the Office Action, the Examiner rejects Claims 85-92 and 101-106 under 35 U.S.C. § 103 as being unpatentable over Salazar et al in view of Brennan, Bastin et al, Liu et al and Fratamico et al.

Specifically, the Examiner states that Salazar et al discloses a method for detecting O157 *E. coli* using oligonucleotide arrays. The Examiner notes that Salazar et al does not teach detecting sequences associated with an *E. coli* O111 isolate. However, the Examiner states that Brennan discloses an array of oligonucleotides that comprise all possible 10-mers; Bastin et al teaches the length of a nucleotide sequence of the O-antigen gene (rfb) cluster as found in *E. coli* O111; Lui et al provides motivation in selecting a sequence from the *wzx* gene as a suitable probe, and teaches that there is "little similarity even at the amino acid sequence level" between various *E. coli* isolates; and Fratamico et al teaches conducting PCR in a wide variety of bacterial strains. Hence, the Examiner concludes that it would have been obvious to modify the method of Salazar et al using sequences specific to *E. coli* O111, and the O157*wzx* gene, which are disclosed by Brennan. Further, the Examiner contends that it would have been obvious to have modified the method of Salazar et al such that those sequences of interest would be employed in an

amplification reaction, e.g., a polymerase chain reaction, as disclosed by Fratamico et al.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

Salazar et al teaches the use of a technique known as nucleic acid scanning by hybridization (NASBH) to identify reliable DNA "signatures" (i.e., identifiers) among different colonies of the same bacterial isolate of enterohemorrhagic *E. coli* serotype O157:H7. The clonal group of bacteria studied had previously been screened using multilocus enzyme (MLEE) analysis and DNA amplification finger-printing (DAF). The NASBH technique was found successful for analyzing closely related bacteria of the O157 serotype.

A key difference between the present invention and the method used by Salazar et al is that in Salazar et al, arbitrarily amplified DNA sequences were analyzed in order to identify DNA sequence patterns common to various types of *E. coli* O157:H7. This is in stark contrast to the present invention where pre-identified genetic sequences are used to identify bacterial serotypes of *E. coli* and *S. enterica*.

Additionally, the oligonucleotide primers used by Salazar et al were not based on any gene sequence and were not designed to have any degree of specificity for particular genes present in the bacterium. These primers, which were not designed to amplify any particular gene in the bacterium, randomly amplify many different genetic sequences, and in related bacterium they often bind the same genetic sequences. Thus, the resulting amplification patterns have a high degree of

similarity. This class of PCR testing is usually called random amplified polymorphic DNA (RAPDS). It is commonly known that RAPDS has technical problems in that the PCR reaction is difficult to control due to the low stringency conditions used, and thus reproducible patterns are difficult to achieve.

Had the methods of Salazar et al been employed on sample containing *E. coli* strains that were unpurified (e.g., a sample also containing serotype O55:H7, the parent of the O157:H7 clone), then it would have produced highly related patterns that would have been difficult to differentiate.

This is a stark contrast to the methods of the presently claimed invention, which can be employed to identify specific serotype(s) when other serotypes of the same genus are present.

Accordingly Applicants respectfully submit that the present invention is not taught or suggested in Salazar et al, and for the following reasons, it is clear that Brennan, Bastin et al, Liu et al and Fratamico et al do not provide the deficiencies that exist therein.

The Examiner contends that Brennan discloses "every 10-mer probe and primer encompassed by the Applicants' method". However, this is an erroneous analysis because while Brennan discloses methods of identifying amino acid sequences that bind to a biologically active macromolecule it does not disclose the sequences of the probes and primers themselves. Rather, Brennan simply invites the skilled person to set up a research program to identify suitable primers and oligonucleotides that bind to biologically active molecules.

More specifically, Brennan merely provides a method of generating every possible 10mer probe for detecting DNA sequences. This is a simple, mathematical permutation that can be used in relation to any probe or primer. This teaching is in the context of a method for conducting a large number of different chemical reactions on a support surface, including antigen-antibody interactions and oligonucleotide binding reactions. The support surface with attached 10mer binding sequences is exemplified for use in confirming the nucleotide sequence of a labeled polynucleotide of interest, which binds to one or more of the particular 10mer capture polynucleotides on the support surface.

The Examiner appears to consider the disclosure of how to create a 10mer sequence of any variation relevant to the present invention. However, Applicants respectfully submit that the skilled person would not take this teaching and combine it with any of the other cited references, and devise any of the methods of the present invention. As 10mer capture probes are short, it is known that they can cross-react with may different oligonucleotide sequences. Applicants have identified unique identifier sequences that differ between bacterial serotypes, said serotypes sharing many highly related genomic sequences. The identifier sequences found by Applicants may thus, be used to detect the presence of specific serotypes in a sample.

Applicants respectfully submit that the skilled person could not possibly be motivated to use the brief description of how to make any 10mer oligonucleotide probe in the analysis of a

sample, in order to detect specific pathogenic bacterial serotypes as claimed in the present invention.

Nonetheless, in order to further distinguish the presently claimed invention over Brennan, 10mer probes and primer have been excluded from the scope of the presently claimed invention. The minimum length for the probes and primers has been increased to 17mers. Support for this amendment can be found in Tables 4-8, which disclose a considerable number of 17mer oligonucleotide probes and primers. The specificity provided by 17mer probes and primers is outlined in the above-referenced book chapter by Rybicki.

Brennan does not teach or suggest the presently claimed invention, even if combined with Salazar et al, as neither teaches the claimed oligonucleotides which are specific for the O-antigens.

As to Bastin et al, Bastin et al was co-authored by one of the inventors of the present application. This document discloses that a specific polysaccharide (GDP-colitose) has a specific set of genes associated with it (synthesizing enzymes) on the O antigen cluster of an *E. coli* serotype O111. Bastin et al merely mentions that certain structures, such as 3,6-dideoxyhexose colitose, have a sugar that is common to the O antigen of *E. coli* and *S. enterica* genera, and that other moieties of O antigens are common between these two bacterial genera. There is nothing in Bastin et al to motivate the skilled person to use the sequence of the *wzx* region of the O antigen cluster of *E. coli* O11 to identify this bacterium from a sample potentially containing a number of different members of

the *E. coli* genus, particularly as there is no motivation provided regarding equivalent regions in other *E. coli* genus members. Indeed, the only comparison between *E. coli* genus members is between the *wzx* region of serotype O11:M92 and the cps region of serotype K-12, which encodes the K capsular antigen of this genus.

As to Lui et al, this is another publication by one of the inventors of the present application. Lui et al may mention that there is little amino acid sequence identity between the *wzx* gene across members of the *E. coli* genus and also the *S. enterica* genus. However, there is nothing in Lui et al to suggest any particular region of the *wzx* gene could possibly be used as a probe or primer. Given the unpredictable nature of the field of biotechnology, it would not have been a matter of routine for the skilled person to select specific regions of the *wzx* gene that could possibly serve as distinguishing regions among serotypes of these bacterial genera. Rather, the focus of Lui et al is the function of a *wzx* gene of *S. enterica* LT2 strains. The key finding of Lui et al was that this gene encodes a flippase enzyme that translocates the O-unit across the cytoplasmic membrane of *S. enterica*.

Thus, while Lui et al may disclose the *wzx* gene, it does not teach or suggest to one skilled person that this gene may be a suitable target for identifying *E. coli*, especially as the oligonucleotides employed in the presently claimed invention are not disclosed in Lui et al.

As to Fratamico et al, this reference teaches vastly different primers for PCR analysis of various antigenic

bacterial strains. The primers are directed to regions that are not related to the O-antigen of the bacteria, as per the oligonucleotides of the presently claimed methods (the secondary oligonucleotides optionally employed in the present invention are directed towards a non-specific region of the bacterium of interest). The sequences used by Fratamico et al are directed to very different regions of the genome (i.e., from DNA sequences which are present in "most", not all, enterohemorrhagic *E. coli*, serotype O157:H7 (see the Abstract thereof).

In contrast, the method of the present invention reliably identifies all groups of serotype O157:H7, *inter alia*, by use of sequences involved in the formation of the O antigen. Such broad-spectrum identification of antigenic serotypes is simply not possible using the methods of Frantamico et al.

The Examiner should be reminded that when considering whether the presently claimed invention is obvious, it is impermissible to use hindsight.

Applicants submit that that the skilled person would not think to combine the vague disclosure of Brennan as to how to make any 10mer oligonucleotide (not for any purpose related to detecting bacteria in a sample), with the NASBH random primer fingerprinting method of Salazar et al. One skilled in the art would not modify the combination of Brennan and Salazar et al. Again, the probes and primers used in the claimed methods have a minimum length of 17mers. As mentioned above, Brennan does not disclose any sequences specific to either of the aforementioned bacterium. Rather, Brennan discloses a technique for generating

all possible 10mer polynucleotide sequences, with no mention of *E. coli* O111 and O157 *wzx* genes. Hence, the skilled person would not think to use the sequences disclosed by Brennan in a PCR reaction for identification of "most" forms of one serotype of pathogenic *E. coli* bacterium O157:H7, as per Fratamico et al.

Accordingly Applicants respectfully submit that the present invention is not taught or suggested in Salazar et al, alone or when combined with the teachings of Bastin et al, Brennan, Liu et al and Fratamico et al, and thus request withdrawal of the Examiner's rejection.

Applicants note that, Claims 93-100 (which correspond to new Claims 115-122) have not been included in this rejection, and thus would appear to be allowable over the prior art.

In view of the amendments to the claims and the arguments set forth above, reexamination, reconsideration and allowance are respectfully requested.

The Examiner is invited to contact the undersigned at his Washington telephone number on any questions which might arise.

Respectfully submitted,

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